

Advanced Concepts in Turfgrass Nutrition

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Justus von Liebig, a mid-nineteenth century chemist known as the "Father of Agricultural Chemistry," postulated that exhausted soil was simply the result of mineral nutrient removal. Based on his philosophy, the giant mineral fertilizer industry was developed. Because of von Liebig's hypothesis, it is frequently assumed that specific levels of mineral nutrients in plant tissues reflect plant performance as well as nutrient availability in the soil. However, the mineral content of individual plants of the same cultivar which exhibit different growth responses is relatively small (Driessche and Wareing, 1966). Also, the rate at which grass leaves grow in response to fertilization is poorly correlated with turfgrass quality (Mehall et al., 1984). It has been observed that vigorously growing turfgrass managed under a high nitrogen regime is often more susceptible to environmental stress than slow growing turfgrass (Green and Beard, 1969; Funk et al., 1967).

Various environmental stress factors influence the onset and rate of senescence (aging) in turfgrasses. Senescence is enhanced by pest attack, high temperature, low light intensity, low or high soil moisture, anaerobic soil conditions, nutrient excess, nutrient deficiency, and toxicity of pesticides (Couch, 1995).

Clipping production of cool-season turfgrasses is generally greatest in spring, followed by a depression during mid-summer (Landschoot and Waddington, 1987; Woolhouse, 1981). Hull (1992) demonstrated that this seasonal pattern is not eliminated by elevated N fertilization. He speculated suppression or inhibition of summer growth in cool-season grasses also may be a response to hormonal change. This seems reasonable, as all plant growth aspects are influenced by hormones.

Mineral Fertilization Impact on Growth and Organic Substances in Turfgrasses

Since early 1960, turfgrass researchers have been studying the relationship between mineral nutrition and the concentration of plant organic substances. Their objective has been to understand the relationship between effective fertilization and seasonal changes. It has been documented that, during the growing season, elevated nitrogen fertilizer levels have a negative impact on root growth of cool season grasses (Adams et al., 1974; Goss and Law, 1967; Madison, 1962). Under such conditions, carbohydrates in storage organs are used to stimulate vegetative shoot growth and thereby decrease the partitioning of energy available for root production (Mifflin, 1980). During winter, when shoot growth and respiration are depressed because of low temperatures, the products of photosynthesis are shifted from the leaves to sites of non-structural carbohydrate storage and enhancement of root development. Powell et al. (1967) reported that N applied to turf during late fall initially inhibited root growth, but subsequent root development was stimulated. Apparently this was due to increased photosynthate accumulation associated with early spring green-up, resulting from elevated chlorophyll synthesis promoted by winter nitrogen availability.

Environments favoring rapid growth often reduce the non-structural carbohydrate content of cool-season grasses (Hull, 1992). Watschke et al. (1972), in a study involving ten Kentucky bluegrass cultivars, showed that those cultivars high in non-structural carbohydrates after four weeks at elevated temperatures were also high in apparent photosynthesis and foliar yield. Turfgrass tolerance to heat stress in July has been related to carbohydrate reserves by others (Wehner et al., 1985; Schmidt and Blaser, 1967). It has also been observed that elevation of nitrogen fertilization reduces heat tolerance of cool season turfgrass due to low levels of carbohydrate reserves in the leaves of vigorously growing plants (Watschke et al., 1972; Pellet and Roberts, 1963; Wehner and

Watschke, 1981). Exposing turfgrasses to low night temperatures improved growth of plants subjected to high day temperatures (Schmidt and Blaser, 1967; Watschke et al, 1970). This was also correlated with an elevated carbohydrate content in the grass.

Wehner and Watschke (1984) suggested that net protein synthesis in cool-season turfgrasses is a heat sensitive process. Heat stress may result in the denaturation of proteins, alteration of membrane fluidity and permeability, and the unfolding of nucleic acid. Heat tolerance of photosynthesis is enhanced by an increase in the number of double bonds (unsaturation) in membrane lipids (Gombos et al., 1994).

Phytohormones Impact Turfgrass Growth

Investigations concerning the control of plant growth by organic chemicals commenced prior to the turn of the century. It was proposed that specific substances controlled cell enlargement and cell division. Charles and Frances Darwin in 1880, demonstrated that light detected by the tip of a grass coleoptile (shoot of a dark-grown seedling) transmits a signal by some means to the lower part, causing the coleoptile to bend. This influence later was identified as organic compounds synthesized in one part of the plant and translocated to another. These compounds, now called hormones, cause physiological responses at very low concentrations. The concept that hormones significantly impact plant growth was initiated in this country during the mid-1930's (Skoog, 1994). A prevalent skepticism concerning the existence of plant-growth-promoting hormones persisted and was exhibited at the time when Fritz Went, in 1935, was unable to demonstrate the *Avena* coleoptile curvature tests for auxins in Pittsburgh, which at that time was severely air polluted. The negative attitude Dr. Went met prompted him to investigate the effect of air pollution on plant hormonal responses. This led to the Haagen-Smit identification of major toxic compounds in smog for which a National

Medal of Science was awarded. Following this work with auxin, Dr. Folke Skoog, in an arena of much skepticism, was able with co-workers to demonstrate the influence of cytokinin hormones on plant growth stimulation. Although skepticism still prevailed, studies of the biological activity of hormones continued. In the 1950's, it was shown that auxins and cytokinins interact in regulating growth and development of roots, shoots, and flowers. Gibberellins and cytokinins promote leaf formation. This knowledge currently is used by scientists working to develop transgenic plants.

Frankenberger and Arshad (1995) have compiled a comprehensive review of the literature that provides evidence to support the idea that exogenous sources of hormones can influence plant growth and that the physiological effects of these materials cannot be replicated by an equivalent application of mineral nutrients. Many of the compounds capable of regulating plant growth and development have been identified. These are referred to as plant growth regulators (PGRs). When produced endogenously, they are called phytohormones. Both terms relate to the hormones which include auxins, gibberellins, cytokinins, ethylene and abscisic acid (Nagri, 1995).

Although plants are capable of synthesizing phytohormones in response to environmental changes, phytohormone production is enhanced in response to applications of certain materials. Exogenously-applied humic acid, triazole compounds, as well as cytokinin materials can enhance endogenous phytohormone synthesis.

Turfgrass research at Virginia Tech during the last ten years has shown that exogenous applications of cytokinin materials increased the endogenous cytokinin concentrations of grasses (Yan, 1993). Applications of cytokinin materials to turfgrasses have delayed senescence and enhanced tolerance to drought, salinity and nematode stresses. Applications of seaweed, an excellent source of cytokinin material, in combination with auxins applications, have been associated with enhanced root development of various turfgrasses grown under stress environments (Figure 1).

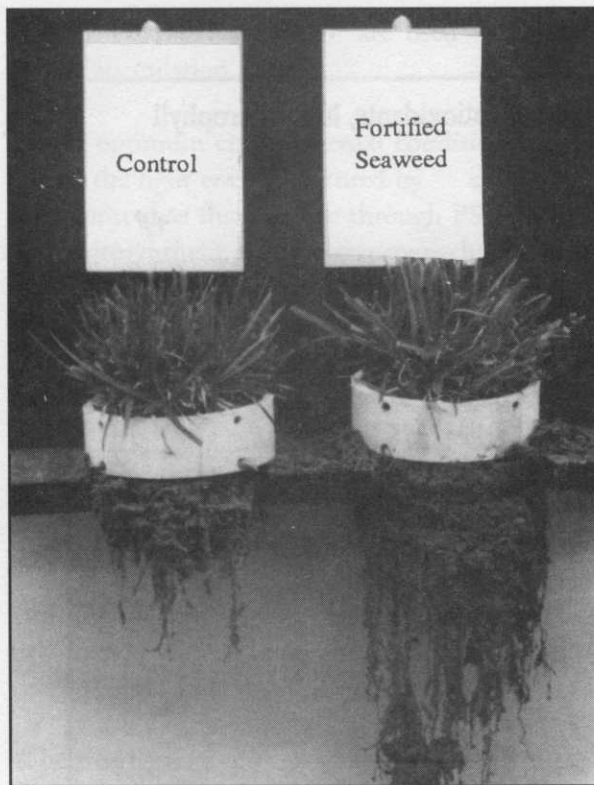


Figure 1. Early spring (1996) root enhancement of September 1995 established tall fescue when treated with fortified seaweed in November 1995.

The influence of phytohormones on the physiological and biochemical mechanisms of plants is complex. Ongoing research has provided some information for explaining the mechanisms involved. Plants may acclimate to stressful environments by changing their membrane composition and fluidity (Hale and Orcutt, 1987). Yan (1993) showed the application of cytokinin-like materials to ryegrass increased membrane fluidity and salt stress tolerance. His results indicate that cytokinins may serve as a signal for membrane modification in plants under stress.

Phytohormones Influence Endogenous Antioxidants

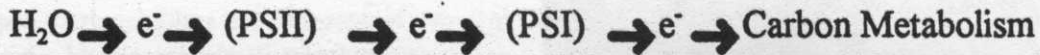
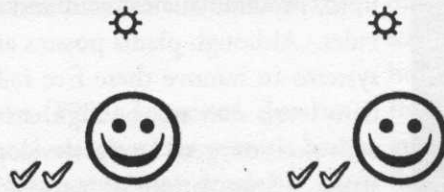
Various environmental stresses (drought, heat, salt, etc.) disturb normal metabolism of plants and result in oxidative stress, characterized by excess accumulation of toxic oxygen free radicals (Larson,

1988; Zhang and Kirkland, 1994). These activated oxygen species, which result when excess electrons generated from photosynthesis or respiration are captured by elemental oxygen (O_2), may damage lipid, protein, nucleic acid and other macromolecules. Although plants possess antioxidant defense systems to remove these free radicals, the antioxidant levels may not be high enough to reduce the activated oxygen levels developed under severe stress. Stimulation of antioxidant activity may condition plants to survive oxidative buildup and improve stress tolerance and growth. Cytokinins can scavenge free radicals directly or prevent the formation of free radicals, as well as serving as a signal for triggering the activity of other antioxidant systems. Mozafar (1994) has reviewed research that shows nitrogen fertilizer may increase, decrease or have no effect on antioxidant content in plants. It appears antioxidants tend to increase with nitrogen supply to a maximum, then decrease with further nitrogen increases. Therefore, N concentration of turfgrass may be considered excessive when additional N fertility causes antioxidant content to decline. This may be complicated, as indicated by numerous reports that show plants supplied with nitrogen from an NH_4^+ source of fertilizer contained less ascorbic acid than when supplied with a NO_3^- source. Also, it has been suggested that excess N fertilization may reduce the allocation of available carbohydrates for antioxidant synthesis. This may explain why actively growing turfgrasses stimulated by high N fertilization are more susceptible to certain environmental stresses.

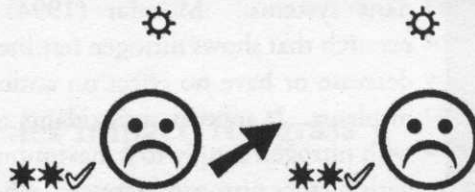
Phytohormones Impact Chlorophyll Fluorescence

One of the major targets for injury caused by environmental stress is the photosynthetic apparatus. Absorbed light energy drives photosynthetic electron transport through photosystem II (PSII) and photosystem I (PSI), leading to the oxidation of water (oxygen evolution), reduction of $NADP^+$ to NADPH, and photophosphorylation generating

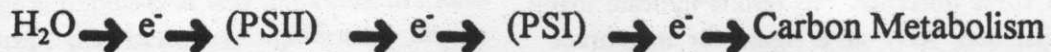
A: Normal senescence, normal stress : Sufficient antioxidants, low chlorophyll fluorescence, normal electron transport.



B: Rapid senescence, high stress: Insufficient antioxidants to offset stress impact--high active oxygen species, high chlorophyll fluorescence, poor electron transport.



C: Delayed senescence, high stressed plants treated with hormones: High antioxidants to offset stress impact--low active oxygen species, low chlorophyll fluorescence, stimulated electron transport.



✓ --- Antioxidant

PSII, PSI --- Photosystem II, Photosystem I.

⊙ --- Energy

* --- Active oxygen species

☺ --- Light capturing pigment complex (normal);

☹ --- Light capturing pigment complex (under stress).

↗ --- Chlorophyll fluorescence

Figure 2 Photosynthetic apparatus as influenced by stress and antioxidant status.

ATP. NADPH and ATP are used for carbon (CO²) assimilation.

Under optimum environmental conditions, about 3% of the light energy captured by chlorophyll is re-emitted as fluorescence through PSII. When the photosynthetic electron transport chain accepts and transfers electrons, chlorophyll fluorescence remains low. When electron carriers are reduced, they cannot accept and transport electrons and the transference of light energy is impaired and chlorophyll fluorescence increases rapidly (Miles, 1990). Any alteration of the photosynthetic apparatus can affect the level of fluorescence. Therefore, the health or capacity of a plant's photosynthetic apparatus or the level of oxidative damage (insufficient antioxidants) can be measured and monitored in a non-destructive way by *in vivo* chlorophyll fluorescence (Figure 2).

When turfgrass is grown under stress, the photosynthetic apparatus will be suppressed by photoinhibition or active oxygen damage. The level of fluorescence then will increase rapidly. Excess N reduces stress tolerance by suppressing antioxidant activity, including cytokinin content. This negative impact of N can be revealed by elevated chlorophyll fluorescence. It is possible to monitor antioxidant status, state of mineral nutrition, and functioning of photosynthetic apparatus by tracing chlorophyll fluorescence kinetics.

Increasing Antioxidants and Lowering Chlorophyll Fluorescence of Turfgrasses

In turfgrass systems, the abiotic or biotic environments are frequently suboptimal; consequently, the grass will experience some sort of stress. Since plant development is influenced by internal as well as external environments, it is possible to influence internal (endogenous) metabolism with the application of external (exogenous) materials.

Research at Virginia Tech has shown that the concentration of antioxidants (alpha-tocopherol, ascorbic acid, 8-carotene, superoxide dismutase) in turfgrasses increased significantly in response to exogenous applications of hormones, particularly auxins and cytokinins.

Applications of seaweed extracts, which contain high levels of cytokinins and auxins (Crouch, 1995), and humic acids, which are rich in auxin activity (Hamence, 1944; O'Donnell, 1973), enhanced antioxidant levels of turfgrasses under both favorable moisture and drought stress conditions thereby stabilizing the synthetic capabilities of the plant. We have observed that treating tall fescue with seaweed extract increased the SOD (antioxidant) activity and lowered chlorophyll fluorescence throughout the growing season.

When the ratio of the variable chlorophyll fluorescence (FV) to the maximum chlorophyll fluorescence (FM) at 690 nm wavelength is calculated, an estimate of photochemical efficiency (FVM=FV/FM) of PSII can be ascertained. Photochemical efficiency may be referred to as chlorophyll activity. From a current field study using urea as the nitrogen source, we demonstrated that non-PGR-treated bentgrass grown under low fertility had higher chlorophyll activity than when grown under high fertility (Figure 3). This corresponded with an increase in concentration of the antioxidant SOD within the leaves. However, under both fertilizer regimes, chlorophyll activity increased when seaweed extract was applied. The antioxidant content of bentgrass grown under low fertility increased only slightly after seaweed extract treatment. On the other hand, the grass grown under the high fertility regime had a three-fold antioxidant (SOD) increase following seaweed extract applications. These results indicate that the antioxidant demand of heavily fertilized bentgrass can be met with exogenous applications of materials containing growth regulating substances.

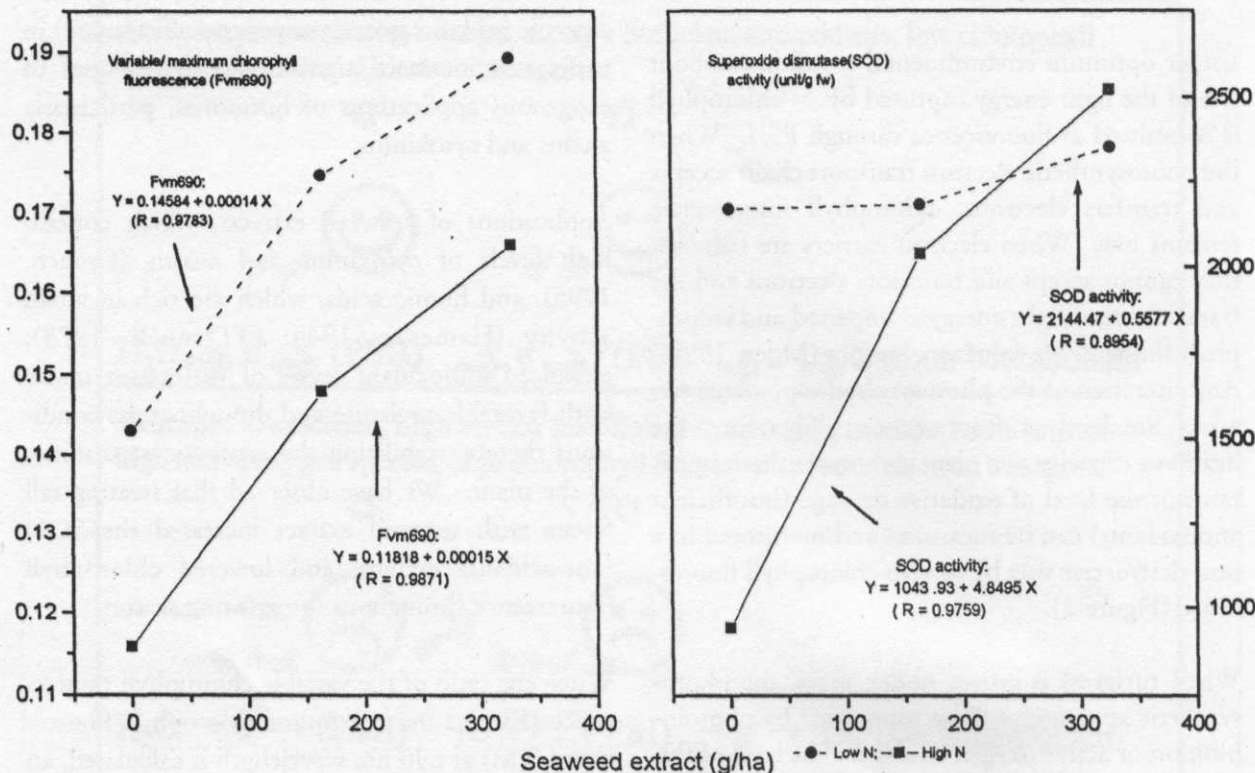


Figure 3 Chlorophyll fluorescence and endogenous antioxidant SOD activity as influenced by Seaweed extract and N level in creeping bentgrass (TRC, 1996)

Application

Current studies are investigating practical means for increasing the efficiency of the photosynthetic apparatus in turfgrasses when exposed to stress. Research has shown that materials such as seaweed extracts and humic acid applied as part of a turfgrass nutritional program stimulate the endogenous production of phytohormones. This, in turn, reduces chlorophyll fluorescence and increases endogenous antioxidants, resulting in greater turf tolerance to environmental stresses. Results obtained show that the use of materials that enhance plant production of phytohormones increases both chlorophyll activity and endogenous antioxidants, which can be used to improve turfgrass growth and development under stress conditions.

Infection and disease development are associated with turfgrass subjected to stress conditions (Nelson, 1996). The greater the level of stress, the greater the fungicide efficacy required to achieve adequate levels of disease control. Therefore, using materials that reduce the impact of environmental stress is a valuable component of integrated pest management (IPM) systems used in the production of turfgrasses. Incorporation of this knowledge into turfgrass systems will enable the turfgrass manager to assess plant health to a much greater extent than currently possible with soil and plant tissue analysis. The turfgrass manager will be able to bring the nutritional status of turf into an advanced dimension.

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Xunghong Zhang has B.S. and M.S. degrees from the Agricultural University of Hebei, People's Republic of China, where he is a professor in the Crop Science Department. He came to Virginia Tech in 1992 as a visiting scholar and now is pursuing his Ph.D. in turfgrass physiology. His current research activities involve antioxidant response of and stress tolerance in turfgrasses.

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Terms to Know

Chlorophyll Fluorescence - light energy surplus to that utilized in photosynthesis (PSII) is dissipated as fluorescence. Fluorescence excited by 690 nm laser diode and passed through a 690 nm filter may be detected by a photodiode.

Endogenous - referring to a natural substance produced within the cells or an organism.

Exogenous - referring to a substance entering the cells of an organism from the outside. Exogenous substances may be natural or synthetic but always are introduced from the external environment of an organism.

Free Radical - a highly reactive chemical containing an unpaired electron. Free radicals are often strong oxidizing agents and are destructive of biological molecules such as enzymes, nucleic acids and membranes.

Hormone - a naturally occurring organic chemical produced in one organ and translocated to another organ where it modifies growth or function. In plants, such chemicals are referred to as phytohormones.

Non-Structural Carbohydrates - carbohydrates involved primarily in energy storage or transport, including soluble sugars, starch and fructans. Structural carbohydrates are those incorporated into the structure of cell walls, including cellulose, hemicellulose and pectins.

Photosynthate - carbon compounds produced directly by photosynthesis. As generally used, the term refers to simple sugars that are transported within green leaf cells and among tissues and organs of a plant.

SOD - Superoxide Dismutase is an enzyme that metabolizes the highly reactive free radical of oxygen, superoxide. It is considered an antioxidant because it eliminates superoxide, a strong oxidizing agent that can damage organic molecular structures.

Unsaturated Lipids - lipid or fat molecules composed of long-chain fatty acids which contain from one to three double bonds between carbon atoms. Such unsaturated lipids are more oxidized and have a lower freezing temperature than saturated lipids. Unsaturated lipids built into the lipid core of membranes make the membrane more fluid during low temperatures and times of stress.